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Oral Administration of the CCR5 Inhibitor, Maraviroc, Blocks HIV *Ex Vivo* Infection of Langerhans Cells within the Epithelium

Journal of Investigative Dermatology (2013) **133,** 2803–2805; doi:10.1038/jid.2013.215; published online 27 June 2013

TO THE EDITOR

Preexposure prophylaxis (PrEP) with oral administration of an antiretroviral is a potential method for preventing acquisition of HIV. A controlled trial in men who have sex with men (the iPrEx trial) showed that daily oral use of tenofovir disoproxil fumarate-emtricitabine (TDF-FTC; Truvada) reduced transmission rates by 44% (Grant *et al.*, 2010). In addition, the HIV Prevention Trial Network (HPTN) 052 trial recently confirmed that antiretroviral treatment leads to 96% reduction in transmission among HIV-negative heterosexual partners of HIV-positive individuals (Cohen *et al.*, 2011). Similar trials, however, with TDF-FTC (the FEM-PrEP trial) or TDF alone (the VOICE trial) were stopped because of poor outcomes (van der Straten *et al.*, 2012). Different results among various trials, which used identical antiretroviral regimens, could be explained by varying compliance with drug use and/or varying drug concentration and activity within the exposed tissue (Patterson *et al.*, 2011).

Langerhans cells (LCs) are $CCR5^+$ dendritic cells located within genital skin and mucosal epithelium (Lederman *et al.*, 2006). In female rhesus macaques exposed intravaginally to simian immunodeficiency virus, up to 90% of initially infected target cells were LCs (Hu *et al.*, 2000). *Ex vivo* experiments with human foreskin explants show that epidermal LCs are target cells for HIV, providing a likely explanation for why circumcision greatly reduces the probability of acquiring HIV (Ganor *et al.*, 2010). LCs also express CD4 and CCR5, but not CXCR4, within the tissue and demonstrate the distinctive characteristics of emigrating from tissue to draining lymph nodes in order to interact with T cells after contact with pathogens (Kawamura *et al.*, 2000). Indeed, epidermal LCs are readily infected *ex vivo* with R5 HIV, but not with X4 HIV, and promote high levels of infection upon interaction with cocultured $CD4^+$ T cells (Kawamura *et al.*, 2000; Ogawa *et al.*, 2013). Thus, LCs probably have an important role in disseminating HIV soon after exposure to virus.

Epidemiologic observations have found that the majority of HIV strains isolated from patients soon after initial infection are R5 HIV strains (i.e., they utilize CCR5; Lederman *et al.*, 2006). Not surprisingly, individuals with homozygous defects in *CCR5* are largely protected from sexually acquiring HIV (Lederman *et al.*, 2006). In addition, three different CCR5-binding topically applied compounds protected female macaques from sexually acquiring simian/human immunodeficiency virus: the N-terminally modified chemokine analog PSC-RANTES, the small-molecule inhibitor CMPD167, and maraviroc (MVC) (Lederman *et al.*, 2006; Veazey *et al.*, 2010). In addition to topical application to vaginal mucosa, oral delivery of CMPD167 protected macaques from vaginal simian/human immunodeficiency virus challenge (Veazey *et al.*, 2005). Given these data, orally administered MVC may prove to be particularly important in PrEP regimens, although its ability to prevent HIV acquisition is unknown.

In the current study, 20 healthy volunteers were randomly divided into four equal groups; they received 300 mg of MVC orally twice daily for 1, 2, 3, or 14 days. To obtain epidermal tissues, all subjects underwent suction blistering of the skin before and 2 hours after the last MVC dose. All subjects had plasma and semen collected 2 hours after their last dose. MVC concentrations in serum, semen, and epidermal tissues were determined by using the liquid chromatography– mass spectometry method, as described previously (Takahashi *et al.*, 2010). Mean concentration ± SD in the epidermis was 21.91 ± 13.80, 23.36 ± 13.28, and 31.54 ± 20.61 nM for individuals taking drug for 1, 2, or 3 days ($n = 5$ for each), respectively. MVC concentrations tended to be higher with a longer dosing period. Consistent with recent data showing high levels of MVC in genital tissue (Dumond *et al.*, 2009), these results indicate that MVC rapidly distributes into the skin at high concentrations. In addition, MVC was detected in semen of all subjects (Supplementary Figure S1 online).

To understand how HIV traverses skin and genital mucosa, an *ex vivo* model was developed whereby resident LCs within epithelial tissue explants are exposed to HIV and then allowed to emigrate from tissue, thus mimicking conditions that occur after mucosal exposure to HIV (Kawamura *et al.*, 2000; Ogawa *et al.*, 2013). In this model, although relatively few productively infected LCs are identified, these cells induce high levels of HIV infection when cocultured with resting autologous $CD4^+$ T cells (Kawamura *et al.*, 2000). In preliminary experiments, HIV infection of LCs, as well as subsequent virus transmission from emigrated LCs to cocultured $CD4^+$ T cells, was decreased in a dose-dependent manner when skin explants were pretreated with various concentrations of MVC before

Accepted article preview online 6 May 2013; published online 27 June 2013

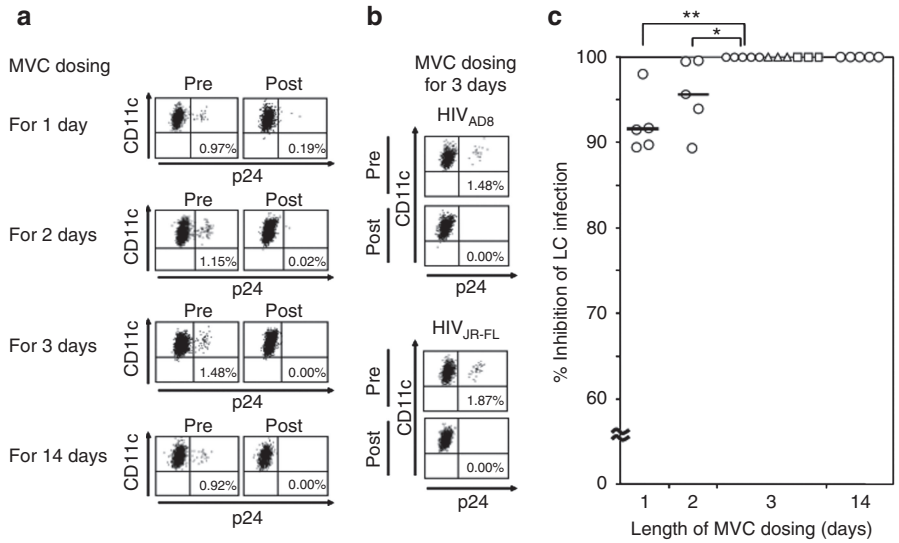

Figure 1. Oral administration of maraviroc (MVC) protects epidermal Langerhans cells (LCs) from *ex vivo* R5 HIV infection. Skin explants were isolated from healthy individuals who had received oral MVC (300 mg twice daily) for the indicated periods of time. These tissues were exposed to HIV_{Ba-L} (**a**, **c**), HIV_{AD8}, or HIV_{JR-FL} (**b**, **c**) and then cultured for 3 days. Emigrated LCs were collected and assessed for HIV infection by flow cytometry. Representative FACS analyses of CD11c and p24 mAb double-stained cells are shown (**a**, **b**). Percent MVC inhibition of LC infection with HIV_{Ba-L} (○), HIV_{AD8} (△), or HIV_{JR-FL} (□) was calculated as described in the text (**c**). *$P<0.05$; **$P<0.01$. Mean values obtained from different donors are shown as horizontal marks.

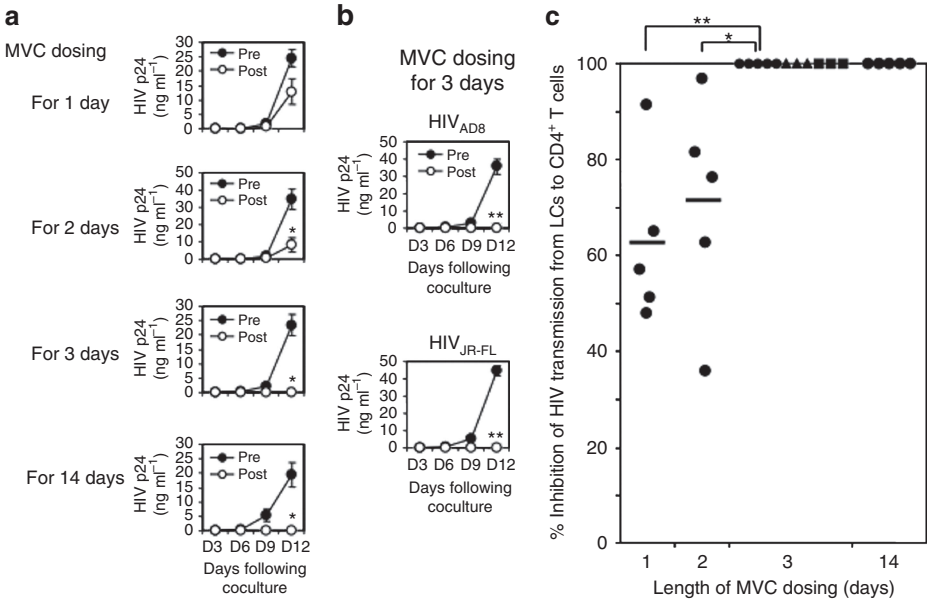

Figure 2. Oral administration of maraviroc (MVC) blocks viral transmission from HIV-exposed Langerhans cells (LCs) to cocultured CD4$^+$ T cells. Skin explants isolated from healthy individuals who had received oral MVC (300 mg twice daily) for the indicated periods of time were exposed to HIV_{Ba-L} (**a**, **c**), HIV_{AD8}, or HIV_{JR-FL} (**b**, **c**), as described in Figure 1. Emigrated LCs were cocultured with autologous CD4$^+$ T cells, and culture supernatants were assessed for p24 content by ELISA. Representative ELISA results are shown (**a**, **b**). Percent MVC inhibition of HIV_{Ba-L} (●), HIV_{AD8} (▲), or HIV_{JR-FL} (■) transmission to cocultured CD4$^+$ T cells was calculated as described in the Methods (**c**). *$P<0.05$; **$P<0.01$. Mean values obtained from different donors are shown as horizontal marks.

HIV exposure (Supplementary Figure S2 online), similar to experiments reported earlier with PSC-RANTES (Kawamura *et al.*, 2004).

Next, the epithelial tissue explants were collected from study subjects after oral treatment with MVC (Supplementary Materials and Methods online). Importantly, oral MVC pretreatment for either 1 or 2 days partially inhibited subsequent *ex vivo* HIV_{Ba-L} infection of LCs within epithelial tissue, whereas MVC administration for either 3 or 14 days completely blocked LCs from *ex vivo* HIV_{Ba-L} infection (Figure 1). These data demonstrate the importance of the length of MVC dosing period before HIV exposure. MVC treatment also consistently prevented HIV_{Ba-L} transmission from LCs to cocultured CD4$^+$ T cells (Figure 2). Furthermore,

MVC administration for 3 days blocked *ex vivo* virus infection of LCs as well as subsequent virus transmission when different R5 HIV strains, HIV_{AD8} and HIV_{JR-FL}, were utilized for an additional six subjects ($n=3$ for each strain, Figures 1 and 2). These data demonstrate that oral administration of MVC for at least 3 days is capable of fully protecting HIV infection of LCs within epithelial tissue.

These experiments provide perhaps the best proof-of-concept test for MVC as a potential PrEP drug, as it would be unethical to expose MVC-treated volunteers to HIV *in vivo*. As proven here, orally delivered MVC rapidly distributes to skin and functionally acts to block infection of relevant target cells, LCs, supporting randomized controlled trials of MVC as a PrEP therapy for individuals at high risk of becoming infected with HIV through sexual exposure.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Kazutoshi Harada and Naotaka Shibagaki for their helpful discussions, and Miyuki Ogino and Naoko Misawa for technical assistance. These studies were supported in part by a grant from the Ministry of Health Science of the Japanese Government (201029002).

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Heterozygous Mutations in *AAGAB* Cause Type 1 Punctate Palmoplantar Keratoderma with Evidence for Increased Growth Factor Signaling

Journal of Investigative Dermatology (2013) **133,** 2805–2808; doi:10.1038/jid.2013.243; published online 11 July 2013

TO THE EDITOR

Punctate palmoplantar keratoderma (punctate PPK or PPKP) is a rare autosomal dominant disorder of keratinization. Three variants of this disease have been described; PPKP1 (OMIM ♯148600, Buschke–Fischer–Brauer type) is characterized by the progressive development of discrete areas of hyperkeratosis on the palms and soles, followed by more extensive diffuse hyperkeratosis on the pressure-bearing areas of plantar skin.

Linkage analyses of families affected by PPKP1 have previously identified a

Abbreviations: AAGAB, gene encoding alpha- and gamma-adaptin-binding protein p34; bp, base pair; OMIM, Online Mendelian Inheritance in Man; PPK(P), (punctate) palmoplantar keratoderma; RTK, receptor tyrosine kinase

Accepted article preview online 6 June 2013; published online 11 July 2013